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A STUDY AND ANALYSIS OF THE EFFECT OF
NUMBERS UPON THE OXYGEN CONSUMP-
TION AND LOCOMOTOR ACTIVITY
OF *CARASSIUS AURATUS*

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1939

By
ARTHUR SHLAIFER

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ARTHUR SHLAIFER

STUDIES IN MASS PHYSIOLOGY: EFFECT OF NUMBERS UPON THE OXYGEN CONSUMPTION AND LOCOMOTOR ACTIVITY OF *CARASSIUS AURATUS*¹

ARTHUR SHLAIFER

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RESEARCH on animal aggregations has shown that many physiological processes of animals are controlled or modified by the number of animals mutually participating in these processes. Allee (1931 and 1934) has comprehensively reviewed the work that has been done in this field. "Group effects" have been found for breeding, hibernation, estivation, growth, death-rate, resistance to toxic agents, and other processes. An effect of grouping on oxygen consumption has been demonstrated by Allee (1927), Schuett (1933 and 1934), and Bowen (1932).

Schuett (1933), attempting to bridge the gap between the solitary fishes and those which exhibit highly integrated mechanisms of aggregation, reported that, in a given volume, the oxygen consumption of isolated goldfishes was significantly higher than if in a group of four. Stating that the unmodified Winkler technique he had used for determining oxygen tension was at fault, owing to nitrite contamination, Schuett (1934) repeated his experiments, using the permanganate modification of the Winkler method, and found no significant difference in oxygen consumption between goldfishes in isolation and in groups of four. Schuett (1934) and Escobar, Minahan, and Shaw (1936) pursued the analysis of the problem by determining the effect of grouping on the locomotor activity of goldfishes; they found a significantly higher rate of activity if the animals were isolated than if they were grouped.

It has been the purpose of the present studies to repeat the analysis of this problem and to find the correlation, if any, between oxygen consumption and locomotor activity.

MATERIALS AND METHODS

The goldfish, *Carassius auratus* Linné, was the experimental animal throughout.

The oxygen consumption was determined by means of the modified Winkler technique, as described by Allee and Oesting (1934). The permanganate modification was used to eliminate the error due to nitrite contamination. Samples of water were taken immediately before and after the test period. These were analyzed for oxygen in terms of cubic centimeters of dissolved oxygen per liter of water. The difference, multiplied by the number of liters of water used and divided by the number of fishes in the group, yields the oxygen consumption per fish.

Heavy mineral oil was used as a seal to prevent the leakage of oxygen from the air into the water during the course of the experiments. The oil seal was approximately 3.5 cm. thick. There is the criticism that mineral oil is not a perfect seal and that oxygen will diffuse through into the water and raise the tension. Control experiments show that this is true. The controls were run with oil that had been used from one to seven times

¹ The writer wishes to express his gratitude to Professor W. C. Allee for the suggestion of this problem and for his helpful advice and criticism.

during a period of $2\frac{1}{2}$ weeks. Fishes in isolation, in groups of two, and in groups of four were allowed to bring down the dissolved oxygen content of the water to the average found at the end of the experiments on fishes in these respective groupings. This assures starting with the usual lower tensions used; and the leaks found are nearly or quite the maximum, not minimum, possible under the given conditions, since the oxygen leak is the greater (other things being equal) the lower the amount of dissolved oxygen in the oil-protected water. Results show a leakage range of from 1.15 per cent to 1.55 per cent of the total oxygen available in both 6 and 12 liters in 3 hours. The greatest leakage occurs at tensions found at the end of the experimental period when four fishes are present. This means that the apparent oxygen consumption of the group of four has been decreased by the leak more than that of the group of two or of the isolated fishes. However, the actual difference in oxygen leaks of the various groups is almost negligible and is not sufficient to alter the conclusions reached in these experiments.

The fishes for each day's tests were picked at random from a lot of twenty selected so as to be approximately equal in size. Their length was between 8 and 10 cm. from the tip of the nose to the base of the tail. Animals of this rather large size were used because (1) one such fish consumes a measurable amount of oxygen in 3 hours; (2) a 3- or 4-hour experimental period is less subject to variations—light intensity, for example—than is a longer period; and (3) the shorter the test period the less the oxygen leak through the oil.

Rectangular, assembled aquariums whose maximum capacities were 7.5 and 15 liters of water were used, the smaller when the experimental volume was 6, the larger when the experimental volume was 12 liters of water in all sets of experiments but two, in which the volume was only 6 liters. The dimensions in centimeters of these aquariums were:

Liters	Length	Width	Depth
7.5.....	25	20	13 (to the 6-liter mark)
15.....	30	27	$\left\{ \begin{array}{l} 8.5 \text{ (to the 6-liter mark)} \\ 17 \text{ (to the 12-liter mark)} \end{array} \right.$

Experiments A, B, C, and D on oxygen consumption were performed at the Hull Zoölogical Laboratory between the hours of 10:00 A.M. and 4:00 P.M. Well water was used at all times. Since the laboratory faces the north, the variations in light intensity were reduced to a minimum during the experimental hours. The temperature range was from 20° to 22° C. Experiments E, F, G, and H on oxygen consumption were performed at the near-by Whitman Laboratory. Since well water was not available, lake water, from which the chlorine and sediment had been removed by filtering through activated charcoal and limestone, was used. Temperature and illumination conditions were approximately the same as at the Hull Laboratory. All experiments performed at Whitman Laboratory were carried out between 12:30 P.M. and 5:00 P.M. The fishes were fed once a week, at the conclusion of a day's experiments, and were not used for at least 20 hours thereafter.

At the end of each day's experiments, the animals were placed into a common aquarium, from which they were picked at random to be placed in isolation, in groups of two, and in groups of four into aquariums containing fresh water through which air was con-

stantly bubbled. These aquariums contained the same volumes of water as were used in the tests the next day. A 20-hour period of acclimatization was allowed in experiments A, B, C, and D and a 21-hour period in Experiments E, F, G, and H. Immediately before each day's tests the air tubes were removed, the water was again changed (except in the "conditioned"-water experiments), and the aquariums were filled 1 liter above the mark. The extra liter of water was added to replace that lost in the process of obtaining three samples of water for analysis at the beginning of the experimental period. These samples were obtained immediately after placing a 3.5-cm. layer of oil on the water. At the close of the test period, samples were again taken and analyzed.

The experiments on locomotor activity were all performed at the Whitman Laboratory under the general conditions mentioned above. The volumes of water, the aquariums, and the feeding and acclimatization techniques were the same as those used in the oxygen-consumption experiments. Also, a 3.5-cm. layer of oil was placed on the water in order to duplicate more completely the general conditions of the experiments on oxygen consumption. In the activity tests the oxygen leak does not enter as a direct factor of error because activity is measured by the observer, not indirectly by chemical analysis. From the twenty fishes used in the oxygen-consumption experiments a group of seven in Experiments A, B, and C and a group of nine in Experiments D, E, F, and G were selected; all the animals selected had distinctive color patterns, so as to be readily identified, and were of approximately the same length.

The technique employed in measuring locomotor activity was that described by Escobar, Minahan, and Shaw (1936) except that all observations were made by one person. Aquariums of the dimensions and capacities listed above were marked off with red India ink into 5-cm. squares on all four sides. Since not all of the dimensions of the aquariums were multiples of five, some of the boxes were rectangles rather than squares. However, movement of a fish into one of these smaller boxes was considered equivalent to movement into the larger squares, since the variable was the same for the various types of grouping. The 5-cm. squares, when viewed in space, constitute a series of imaginary cubes of 125 cc. capacity through which the animals pass when swimming. The eye of the fish was used as the anatomical landmark in judging whether it had passed from one cube into another. The swimming of a fish from one cube into another constitutes a "movement."

There are several sources of error inherent in this method. The greatest lies in the poor estimation of the number of cubes traversed by a rapidly moving animal. When it is moving in several planes at once diagonally across the aquarium, the error is increased. The error can be diminished to some extent by having the guide lines as narrow as possible without obscuring vision.

Another source of error is the fact that fishes may make several movements within a cube; these movements are not recorded in the count. Or they may, by one strong movement, traverse as many as five cubes; and we have no data concerning the oxygen requirements of relatively strong and relatively weak movements which move the animal through the same space. Also, in moving horizontally or vertically from one cube into another, a fish may traverse as many as 9 cm. or as little as 1 cm.

No attempt has been made to differentiate vertical, horizontal, and diagonal movements. The observer is stationed about 2 feet away from the aquarium. It is essential that no sudden noises or movements occur during the observations, as the animals may react violently to them. At no time during the recorded experiments did the fishes

appear to be reacting to such external stimuli. Neither did they give indications of being aware of the presence of the observer. With some practice and the aid of two mirrors placed so as to reflect the narrow sides, the observer facing the long side of the aquarium with his eyes below the level of the water can observe movements in three dimensions, though the accuracy is undoubtedly less than with three observers.

Since the fishes were of distinctive color patterns, individual records could be kept. Each animal was observed for 5 minutes of each experimental hour. Each was placed in a different type of grouping each day so that possible variations might be more evenly distributed. Time was recorded by a stop watch.

OXYGEN CONSUMPTION

Oxygen tension is an important factor in experiments involving oxygen consumption of aquatic animals that utilize dissolved oxygen, and three general categories are recognized (Hyman, 1929): (1) animals completely dependent on the oxygen tension of the water (the lower the tension the less the respiratory rate; the higher the tension the greater the respiratory rate); (2) animals independent of the oxygen tension at the median ranges and dependent at the extremes; and (3) animals independent of the oxygen tension of the medium. Keys (1930) says that no general statements can be made for fishes and that at the asphyxial level their oxygen consumption is very low. Toryu (1927) concludes that *C. auratus* is independent of the oxygen tension of the water between 7.5 and 2 cc. of oxygen per liter. This would indicate that it belongs in class (2).

By correlating the oxygen tension at the end of an experiment with the total oxygen consumption of the fishes, it was possible to determine that the animals in the present set of experiments were independent of the oxygen tension from 7.0 to 1.5 cc. of oxygen per liter. Below this the oxygen-consumption rate was lower and drifted with the tension. The results of the oxygen-consumption experiments listed are those in which the oxygen tension at the conclusion of the experiment was 1.5 cc. per liter or higher.

In Experiments A-D of Table 1, each figure for the oxygen consumption per fish in a group of four is an average of the consumption of the four animals in that experiment. Similarly, the consumption per fish in a group of two is the average of the consumption of the two fishes. Each figure for the oxygen consumption of an isolated animal does not represent an average of four or of two fishes, each in isolation, but is the observed respiration by one. Hence, Experiments A-D in Table 1 do not represent an equal sampling. The fishes in a group of four have had the greatest sampling, and those in isolation the least. Experiments E-H of Table 1 do have equal sampling, since there are four times as many cases listed for isolated fishes as for those in a group of four and twice as many as for those in a group of two.

Tables 1 and 1a summarize the effect of grouping on the oxygen-consumption of the goldfish. In general, these tables indicate that isolated fishes consume more oxygen than do those in a group of two or four, and that fishes in a group of two tend to consume the same amount of oxygen as do those in a group of four. In some cases, e.g., B and F in the "I vs. II" column and H in the "I vs. IV" column in Table 1a, the differences lack statistical significance. Conditions B and C in the "II vs. IV" column of Table 1a indicate that fishes in a group of two have a significantly higher oxygen-consumption rate than do animals in a group of four. The combined experimental means show very good significance for the "I vs. II" and "I vs. IV" relationships, while the "II vs. IV"

relationship shows no statistical significance, though the *P* value is close to the upper limit of accepted statistical significance.

TABLE 1

EFFECT OF NUMBERS PRESENT UPON THE AMOUNT OF OXYGEN CONSUMED PER FISH
BY GROUPED AND ISOLATED GOLDFISHES UNDER EXPERIMENTAL CONDITIONS

EXPERIMENTAL CONDITION	TEST PERIOD (HOURS)	I. ONE FISH		II. TWO FISH		IV. FOUR FISH	
		No. of Cases	Mean Cc. O ₂ Con- sumed per Fish	No. of Cases	Mean Cc. O ₂ Con- sumed per Fish	No. of Cases	Mean Cc. O ₂ Con- sumed per Fish
A ¹ , 3, 4, 6,	4	12	9.21	15	5.67	10	4.64
B ¹ , 4, 6,	4	18	8.46	19	8.43	8	5.80
C ¹ , 5, 6,	4	20	9.45	20	7.28	11	5.69
D ² , 5, 6,	4	34	12.36	34	8.17	24	8.24
E ¹ , 4, 6,	3	20	7.85	10	5.82	5	5.07
F ² , 5, 6,	3	20	8.76	10	7.02	5	6.18
G ¹ , 4, 7,	3	12	6.50	6	3.91	3	3.72
H ² , 5, 7,	3	12	7.54	6	3.54	3	4.15
Average of com- bined means.		8	8.77	8	6.23	8	5.44

¹ Six liters of water.

³ Preliminary set.

⁵ In 15-liter aquarium.

⁷ "Conditioned" water.

² Twelve liters of water.

⁴ In 7.5-liter aquarium.

⁶ Unconditioned water.

TABLE 1a

STATISTICAL ANALYSIS OF TABLE 1 SHOWN IN *P* VALUES*

Experimental Condition	I vs. II	I vs. IV	II vs. IV
A.	.0028	.0020	.0800
B.	None†	.0476	.0044
C.	.0002	.0000	.0003
D.	.0004	.0000	None†
E.	.0148	.0160	.1216
F.	.0640	.0370	.4000
G.	.0040	.0250	.6620
H.	.0010	.0950	.4780
A-H combined†.	.0012	.0000	.0566

* Upper limit of significance is set at .05. This is three times the probable error. A *P*-value of .0010 indicates good significance, while a value of .1000 indicates no significance ("Student," 1925).

† "None" indicates that no significance was calculated, since the means are almost identical.

‡ In the combination of A-H in Table 1a and A-G in Table 2a it must be noted that in some cases the oxygen consumption and locomotor activity of the fishes are significantly affected by differences in the quantity and quality of the water (see Tables 4-9). However, the combinations are justified since we are dealing with sets of paired data.

LOCOMOTOR ACTIVITY

Tables 2 and 2a summarize the effect of grouping on the locomotor activity of the goldfish. The general technique used has been described above. There has been equal

sampling for the fishes in each type of grouping, since individual records have been kept. Each animal has been in each type of grouping, in any given experiment, for the same period of time. The test periods were 3 and 4 hours, with each animal under observation for 5 minutes of each hour of that time.

TABLE 2

EFFECT OF NUMBERS PRESENT UPON THE ACTIVITY OF GROUPED AND ISOLATED GOLDFISHES UNDER EXPERIMENTAL CONDITIONS (RESULTS ARE IN TOTAL AMOUNT OF MOVEMENT* PER FISH PER 5-MINUTE PERIOD OF OBSERVATION)

EXPERIMENTAL CONDITION	TEST PERIOD (HOURS)	I. ONE FISH		II. TWO FISH		IV. FOUR FISH	
		No. of Cases	Mean Activity	No. of Cases	Mean Activity	No. of Cases	Mean Activity
A ¹ , 3, 5, 7,	4	7	82.9	7	48.0	7	54.8
B ¹ , 4, 5, 7,	4	7	55.0	7	47.2	7	41.8
C ² , 4, 5, 7,	4	7	76.7	7	48.9	7	45.4
D ¹ , 3, 5, 7,	3	9	137.8	9	76.1	9	74.9
E ² , 4, 5, 7,	3	9	154.0	9	76.7	9	65.7
F ¹ , 3, 6, 8,	3	9	136.8	9	81.1	9	61.5
G ² , 4, 6, 8,	3	8	163.5	8	78.3	8	73.9
Average of com- bined means.		7	115.2	7	65.2	7	59.7

* See discussion of locomotor activity under "Materials and Methods" for explanation of term "movement."

¹ Six liters of water.

² In 7.5-liter aquarium.

³ Unconditioned water.

⁴ Twelve liters of water.

⁵ In 15-liter aquarium.

⁶ "Conditioned" water.

⁷ Each case is the average of twelve 5-minute observation periods.

⁸ Each case is the average of six 5-minute observation periods.

TABLE 2a

STATISTICAL ANALYSIS OF TABLE 2 SHOWN IN *P*-VALUES*

Experimental Condition	I vs. II	I vs. IV	II vs. IV
A.	.0170	.0110	.2200
B.	.3700	.0004	.3560
C.	.0390	.0228	.3700
D.	.0204	.0102	.8770
E.	.0400	.0220	.1964
F.	.0820	.0266	.0758
G.	.0678	.0454	.6160
A-G combined†.	.0030	.0032	.1700

* See Table 1a for discussion of *P*-value.

† See Table 1a for discussion of combination of experimental means.

The experiments on locomotor activity were performed to see whether the "group effect," as manifest in the oxygen-consumption experiments, is correlated with the amount of movement performed by each fish. Since, in general, oxygen consumption is directly proportional to activity, the locomotor-activity experiments should give results similar to those on oxygen consumption.

Tables 2 and 2a indicate, in general, that isolated fishes have a higher rate of locomotor activity than do those in a group of two or a group of four, and that fishes in a group of two tend to have the same rate of locomotor activity as do those in a group of four. Thus, the results correlate, as they should, with the results on oxygen consumption. In several cases, e.g., B, F, and G in the "I vs. II" column of Table 2a, the differences lack statistical significance. The combined experimental means show good significance for the "I vs. II" and the "I vs. IV" relationships, and a *P*-value considerably above the upper limit of accepted statistical significance for the "II vs. IV" relationship.

Since these observations on locomotor activity were made over a period of 3 or 4 hours with an oil seal, and since no Winkler analyses were made for oxygen tension at

TABLE 3

COMPARING THE ACTIVITY OF FISHES IN A GROUP OF FOUR DURING THE FIRST AND LAST HOURS OF THE EXPERIMENTAL PERIOD (RESULTS ARE IN TOTAL AMOUNT OF MOVEMENT* PER FISH PER 5-MINUTE PERIOD OF OBSERVATION)

EXPERIMENTAL CONDITION †	TEST PERIOD (HOURS)	FIRST HOUR		LAST HOUR		<i>P</i> -VALUE‡ FIRST VS. LAST
		No. of Cases	Mean Activity	No. of Cases	Mean Activity	
A†.....	4	7	66.7	7	51.0	.0840
B‡.....	4	7	44.0	7	37.2	.1800
C‡.....	4	7	49.7	7	45.0	.4680
D§.....	3	9	77.1	9	75.2	None**
E§.....	3	9	64.1	9	63.1	None**
F 	3	9	65.0	9	55.8	.2480
G 	3	8	70.9	8	72.2	None**
Average of combined means††.....		7	62.5	7	57.1	.0348

* See discussions of locomotor activity under "Materials and Methods" for explanation of term "movement."

† Letters correspond to those in Table 2.

‡ Each case is the average of three 5-minute observation periods.

§ Each case is the average of four 5-minute observation periods.

|| Each case is the average of two 5-minute observation periods.

¶ See Table 1a for discussion of *P*-value.

** "None" indicates that no significance was calculated, since the means are almost identical.

†† See Table 1a for discussion of combination of experimental means.

the end of the test period, the criticism might be made that the fishes in the group of four might have been reacting to an oxygen tension below 1.5 cc. per liter during the latter part of the test period. If so, the results obtained could be attributed not to a "group effect" but to a low oxygen tension which inhibits respiration and activity.

Accordingly, the movements of fishes in a group of four during the first hour of the test period, when the oxygen tension is the highest, were compared with the movements during the last hour of the test period, when the oxygen tension is the lowest. The results are summarized in Table 3.

In no test was the activity of the fishes significantly higher during the first hour of the test period. However, a consideration of the combined means does show a signifi-

cantly higher rate of activity during the first hour, as compared with that of the last hour. When, however, this is compared with the larger mean differences in Table 2, it can be seen that the factor of inhibiting oxygen tensions is not the principle one at work. In fact, under the conditions of these experiments, it is reasonable to assume that the lower activity during the last hour of the test period indicates a more complete recovery from the effect of handling incident to manipulations near the beginning of the observations, rather than being primarily a result of lessened oxygen supply.

When isolated fishes and those in a group of four are compared, the "group effect" on their oxygen consumption and locomotor activity is apparent. Isolated animals are more active and have a higher rate of oxygen consumption. The only exception to each individual comparison being significant when considered alone is found in H of Table 1*a*, and there, we believe, the lack of significance is due to the small number of tests for the group of four. Supporting this view is the marked significance when A-H are combined.

The group of two seems to occupy the same place as the group of four in the comparison of isolated and grouped fishes. There are more cases where individual experiments fail to show that the higher oxygen consumption and locomotor activity of the isolated animal is significant when compared with a fish in a group of two, but the combined means of all the experiments indicate good statistical significance. In general, the fishes in groups of two tend to have the same rate of oxygen consumption and locomotor activity as those in groups of four. Such results may indicate that the isolated state in the phenomenon is a unique one.

VOLUME RELATIONS

Two important factors that enter into the analysis of the problem are the volume of water available to each animal and the dimensions of the container. Schuett (1933) states that when the volume of water per fish is equal the "group effect" on oxygen consumption disappears. Escobar, Minahan, and Shaw (1936) state that "when a fish is limited to a given volume of water the configuration of that volume or the interrelation between the relative magnitudes of its three dimensions determines to a considerable extent the total amount of movement." They find that when goldfishes in isolation and in groups of four are placed in four different volumes of water—1,625 cc., 3,250 cc., 4,875 cc., and 6,500 cc.—minimal activity in grouped and isolated animals occurs at a volume of 4,875 cc., having a depth of 16.92 cm., a width of 12 cm., and a length of 24 cm. The width and length are constant for all volumes. "The locomotor mechanisms of fishes (with a few exceptions, e.g., seahorse) are adapted to propel the fish along the long axis of its body, the latter being normally oriented in most species of fishes in a horizontal plane," say Escobar, Minahan, and Shaw (1936).

Two questions involved in the volume-relationship analysis are: (1) Does the oxygen consumption and locomotor activity of a goldfish vary with the volume of water available to it? (2) When the volume of water per fish is equal, does the "group effect" on oxygen consumption and locomotor activity disappear? Tables 4-7 attempt to answer these questions, utilizing the data in Tables 1 and 2. Only those data were used with which legitimate paired comparisons could be made.

Table 4 indicates that, in the cases tested, a goldfish has a higher rate of oxygen consumption when in a larger volume of water. Table 6, the corresponding table on locomotor activity, should confirm the results of Table 4 but does not. An increase in volume does not produce an increase in the activity of a goldfish. This contradiction

TABLE 4

COMPARING THE OXYGEN CONSUMPTION OF FISHES IN ISOLATION, GROUPS OF TWO, AND GROUPS OF FOUR IN 6 LITERS WITH THE OXYGEN CONSUMPTION OF SIMILAR COMBINATIONS IN 12 LITERS*

SIX LITERS		TWELVE LITERS	
Grouping	Mean Cc. of O ₂ Consumed per Fish	Grouping	Mean Cc. of O ₂ Consumed per Fish
Isolated.....	$\begin{cases} 8.46 \\ 7.85 \\ 6.50 \end{cases}$	Isolated.....	$\begin{cases} 12.36 \\ 8.76 \\ 7.54 \end{cases}$
Group of two.....	$\begin{cases} 8.43 \\ 5.82 \\ 3.91 \end{cases}$	Group of two.....	$\begin{cases} 8.17 \\ 7.02 \\ 3.54 \end{cases}$
Group of four.....	$\begin{cases} 5.80 \\ 5.07 \\ 3.72 \end{cases}$	Group of four.....	$\begin{cases} 8.24 \\ 6.18 \\ 4.15 \end{cases}$
Mean.....	6.17	Mean.....	7.33

Statistical significance of the difference, .0236

* Figures are based on data in Table 1.

TABLE 5

COMPARING THE OXYGEN CONSUMPTION OF FISHES IN ISOLATION OR GROUPS OF TWO IN 6 LITERS WITH THAT OF FISHES IN GROUPS OF TWO OR FOUR IN 12 LITERS WHEN THE VOLUME OF WATER PER FISH IS EQUAL*

SIX LITERS		TWELVE LITERS	
Grouping	Mean Cc. of O ₂ Consumed per Fish	Grouping	Mean Cc. of O ₂ Consumed per Fish
Isolated.....	$\begin{cases} 8.46 \\ 7.85 \\ 6.50 \end{cases}$	Group of two.....	$\begin{cases} 8.17 \\ 7.02 \\ 3.54 \end{cases}$
Group of two.....	$\begin{cases} 8.43 \\ 5.82 \\ 3.91 \end{cases}$	Group of four.....	$\begin{cases} 8.24 \\ 6.18 \\ 4.15 \end{cases}$
Mean.....	6.83	Mean.....	6.22

Statistical significance of the difference, .2838

* Figures are based on data in Table 1.

makes a generalization impossible but may mean that, as used, the oxygen-consumption tests are the more sensitive.

Table 5 shows a disappearance of the "group effect" on oxygen consumption when the volume of water per fish is equal. Table 7, the corresponding table on locomotor activity, again contradicts the results of the table on oxygen consumption. Thus, it indicates that the smaller groups in the smaller volumes are the more active. However, if both Tables 5 and 7 are examined critically, it is found that the isolated fishes have a considerably higher rate of oxygen consumption and locomotor activity than do those

TABLE 6

COMPARING THE ACTIVITY OF FISHES IN ISOLATION, GROUPS OF TWO, AND OF FOUR IN 6 LITERS WITH THE ACTIVITY OF SIMILAR COMBINATIONS IN 12 LITERS*

SIX LITERS		TWELVE LITERS	
Grouping	"Movements"† per Fish per 5-Minute Period of Ob- servation	Grouping	"Movements"† per Fish per 5-Minute Period of Ob- servation
Isolated.....	$\begin{cases} 82.9 \\ 137.8 \\ 136.8 \end{cases}$	Isolated.....	$\begin{cases} 76.7 \\ 154.0 \\ 163.5 \end{cases}$
Group of two.....	$\begin{cases} 48.0 \\ 76.1 \\ 81.1 \end{cases}$	Group of two.....	$\begin{cases} 48.9 \\ 76.7 \\ 78.3 \end{cases}$
Group of four.....	$\begin{cases} 54.8 \\ 74.9 \\ 61.5 \end{cases}$	Group of four....	$\begin{cases} 45.4 \\ 65.7 \\ 73.9 \end{cases}$
Mean.....	83.8	Mean.....	87.0

Statistical significance of the difference, .4982

* Figures are based on data in Table 2.

† See discussion of locomotor activity under "Materials and Methods" for explanation of term "movement."

in the group of two in twice the volume. On the other hand, the fishes in the group of two in both tables have a rate of oxygen consumption and locomotor activity that corresponds closely to that of the animals in the group of four in twice the volume. This again may mean that the isolated state is a unique one and is sufficient in these cases to overcome the handicap of a reduced total volume (the volume per fish being the same) in manifesting its effect on oxygen consumption and locomotor activity. Evidently, the relation of the volume of water per fish and the dimensions of the container to oxygen consumption and locomotor activity is a complex which requires further analysis.

Schuett (1934), using goldfishes 3.5-4.0 cm. in length, found minimal locomotor activity in a 7.5-liter aquarium in a group of four and in a 15-liter aquarium in a group

of eight. Thus, in the smaller aquarium the activity of a group of eight was higher than that of a group of four even though less water was available, per fish, to those in the larger group.

Escobar, Minahan, and Shaw (1936), working with goldfishes in isolation and in groups of four, found not a regular increase of activity with an increase in the volume per fish but a volume at which minimal activity occurred.

No minimal rates of oxygen consumption and locomotor activity were found in the present studies but might be discovered by using larger groups and larger aquaria.

TABLE 7
COMPARING THE ACTIVITY OF FISHES IN ISOLATION OR GROUPS
OF TWO IN 6 LITERS WITH THAT OF FISHES IN GROUPS OF TWO
OR FOUR IN 12 LITERS WHEN THE VOLUME OF WATER PER FISH
IS EQUAL*

SIX LITERS		TWELVE LITERS	
Grouping	"Movements"† per Fish per 5-Minute Period of Ob- servation	Grouping	"Movements"† per Fish per 5-Minute Period of Ob- servation
Isolated	{ 82.9 137.8 136.8	Group of two	{ 48.9 76.7 78.3
Group of two	{ 48.0 76.1 81.1	Group of four	{ 45.4 65.7 73.9
Mean	93.8	Mean	64.8

Statistical significance of the difference, .0424

* Figures are based on data in Table 2.

† See discussion of locomotor activity under "Materials and Methods" for explanation of term "movement."

"CONDITIONED" WATER

In an attempt to determine whether there was a chemical factor involved in the "group effect," the following procedure was used. The fishes were placed in isolation, groups of two and of four in aquariums in fresh lake water for a 21-hour period of acclimatization prior to the actual test. However, instead of being transferred to fresh lake water again for the tests, as was the procedure in the controls, they were kept in the same water that had been used for acclimatization. This water was called "conditioned" because fishes had lived in it and had added chemical substances to it. The animals used in these "conditioned"-water experiments were fed once a week, but at least 48 hours were allowed to elapse after feeding before any tests were run. According to Allee, Oesting, and Hoskins (1936), "the amount of conditioning is approximately a factor of the number and the size of fishes present, together with the volume of the

water to which they were exposed, and may be indicated by a "conditioning coefficient."² In these experiments, on both oxygen consumption and locomotor activity in "conditioned" water, the coefficients were: 15 and 7.5 for an isolated fish in 6 and 12 liters, respectively; 30 and 15 for a group of two in 6 and 12 liters, respectively; and 60 and 30 for a group of four in 6 and 12 liters, respectively.

The controls were run with fishes placed into fresh lake water for the test period. The animals in the "conditioned"-water experiments were transferred to aquariums serving as temporary receptacles, while an oil film was placed on the "conditioned"

TABLE 8

COMPARING THE OXYGEN CONSUMPTION OF FISHES IN "CONDITIONED" WATER WITH THE OXYGEN CONSUMPTION IN UNCONDITIONED WATER*

"CONDITIONED" WATER		UNCONDITIONED WATER	
Grouping	Mean Cc. of O ₂ Consumed per Fish	Grouping	Mean Cc. of O ₂ Consumed per Fish
Isolated	$\begin{cases} 6.50^\dagger \\ 7.54 \end{cases}$	Isolated	$\begin{cases} 7.85 \\ 8.76 \end{cases}$
Group of two	$\begin{cases} 3.91 \\ 3.54 \end{cases}$	Group of two	$\begin{cases} 5.82 \\ 7.02 \end{cases}$
Group of four	$\begin{cases} 3.72 \\ 4.15 \end{cases}$	Group of four	$\begin{cases} 5.07 \\ 6.18 \end{cases}$
Mean	4.89	Mean	6.78

Statistical significance of the difference, .0030

* Figures are based on data in Table 1.

† Each figure in the "conditioned" column represents the same volume of water as does each corresponding figure in the unconditioned column.

water. They were then replaced into the test aquarium. This procedure assured an equal effect of handling in both controls and experimentals.

The "group effect" in "conditioned" water is listed under G and H in Tables 1 and 1a and under F and G in Tables 2 and 2a. The controls are E and F in Tables 1 and 1a and D and E in Tables 2 and 2a. In general, the results for "conditioned" water coincide with those for unconditioned water as far as the "group effect" is concerned. The isolated animal is found to be the most active and to have the highest rate of oxygen consumption. Tables 8 and 9 contain a direct comparison of oxygen consumption and locomotor activity of fishes in the same types of grouping in "conditioned" and unconditioned waters.

Table 8 indicates that fishes in "conditioned" water have a significantly lower rate

² The conditioning coefficient is obtained by dividing the product of the number of conditioning fishes times their length in millimeters by the number of liters of water conditioned (Allee, Oesting, and Hoskins, 1936).

of oxygen consumption than do those in unconditioned water. The explanation of this result is not clear at this time. Table 9 on locomotor activity shows no difference in

TABLE 9
COMPARING THE ACTIVITY OF FISHES IN "CONDITIONED"
WATER WITH THE ACTIVITY IN
UNCONDITIONED WATER*

"CONDITIONED" WATER		UNCONDITIONED WATER	
Grouping	"Movements"† per Fish per 5-Minute Period of Ob- servation	Grouping	"Movements"† per Fish per 5-Minute Period of Ob- servation
Isolated.....	{ 136.8† 103.5	Isolated.....	{ 137.8 154.0
Group of two.....	{ 81.1 78.3	Group of two....	{ 76.1 76.7
Group of four.....	{ 61.5 73.9	Group of four....	{ 74.9 65.7
Mean.....	99.2	Mean.....	97.5

Statistical significance of the difference, .6718

* Figures are based on data in Table 2.

† See discussion of locomotor activity under "Materials and Methods" for explanation of term "movement."

‡ Each figure in the "conditioned" column represents the same volume of water as does each corresponding figure in the unconditioned column.

TABLE 10
EFFECT OF NUMBERS PRESENT UPON THE AMOUNT OF OXYGEN
CONSUMED BY GROUPED AND ISOLATED GOLDFISHES*

ISOLATED		GROUP OF FOUR		MEAN DIFFERENCE	P- VALUE†
No. of Cases	Mean Cc. of O ₂ Consumed per Fish	No. of Cases	Mean Cc. of O ₂ Consumed per Fish		
40×1	4.60	10×4	3.22	1.38	.0009

* Test period is 3 hours. Volume is 6 liters of unconditioned water in a 7.5-liter aquarium.

† See Table 1a for discussion of P-value.

activity between fishes in "conditioned" and unconditioned water. Again this may mean that, as used, the oxygen-consumption tests are the more sensitive.

PARTIAL RECHECK OF RESULTS, USING MANOMETRIC METHOD

Since it was thought that even the modified method of Winkler, used in determining dissolved oxygen in the oxygen-consumption tests, might possibly contain some hidden error, it was decided to repeat some of the tests, using the Van Slyke manometric method (Van Slyke and Neill, 1924; Oesting, 1934). Allee and Oesting (1934) and Wilder (1937) tested the relative accuracy of the modified Winkler and Van Slyke methods and found agreement.

The materials and methods were the same as those described for the oxygen-consumption experiments using the modified Winkler method. Experimental conditions were identical with those of the earlier series. The oxygen consumption of an isolated fish was compared with that of one in a group of four. The results are summarized in Table 10. The statistical significance of the results is high and shows again that, other things being equal, the oxygen consumption of an isolated fish is higher than that of one in a group of four; this confirms results listed in Table 1. Thus, in so far as tested, findings obtained with the modified Winkler method are confirmed by the Van Slyke manometric method. This agrees with the experience of Wilder (1937).

DISCUSSION

The explanation of the effect of grouping on the respiration and locomotor activity of goldfishes is still hypothetical. Schuett (1933) dismissed the possibility of oxygen tension exerting any effect, at least until the asphyxia level is reached. Toryu (1927) reached the same conclusion, and our own experience is similar. Schuett (1933) also dismissed the possibility that the increased production of carbon dioxide by grouped fishes was the responsible factor.

Escobar, Minahan, and Shaw (1936) offer the suggestion that an isolated fish has no obstruction in its path of motion and can continue along this path undisturbed. On the other hand, a fish in a group must constantly make adjustments to the presence of its neighbors. Though isolated fishes, when active, usually swim in one unobstructed plane, their activity is less constant, and much of it is composed of a series of sudden spurts. Grouped fishes usually swim in several planes; but their activity is much more constant, since they are constantly subjected to stimuli from others in the group.

Vision as an important integrating factor for aggregations of fishes has been demonstrated by Bowen (1931). She reported that blinded catfishes of the species *Ameiurus melas*, which in contrast to *C. auratus* is a more definitely schooling animal, did not aggregate and that normal fishes did not respond in the dark. Bowen (1931) also found that responses to touch are of great importance and that the resulting stimulus may perhaps be the fundamental cause of close aggregations, with vision serving only as a means by which the fishes find one another. Bowen (1932) showed that catfishes in groups of four have a greater rate of oxygen consumption per fish than do those in isolation. This is what would be expected from a closely schooling fish when the individuals push actively toward the center of the group.

Vision may be acting as a factor inhibiting the activity of grouped goldfishes. If so, provided other factors are not exerting an important effect at the same time, blinded goldfishes and goldfishes kept in the dark should not manifest a "group effect." Experiments are now in progress in our laboratory to explore this possibility. It may be that sight reactions will account for all of the observed phenomena of "group effect" in the

goldfish. However, it is possible that vibrations of tails of fishes set up pressure disturbances detected perhaps by lateral line organs, or that other sense organs are involved, either directly or indirectly. Planned experiments include an analysis of these factors.

Our results on oxygen consumption confirm those obtained by Schuett (1933) when there was an error in his technique, and contradict his later results (1934) when the error had been corrected. Schuett (1934) did find a higher rate of locomotor activity for goldfishes in isolation than for those in groups of four. This result does not correlate with his failure to find a higher rate of oxygen consumption in the isolated fish, using the modified Winkler technique. Schuett's experiments on locomotor activity were performed in 7.5- and 15-liter aquariums as were our experiments. His experiments on oxygen consumption were performed in Erlenmeyer flasks; and Schuett offers a possible explanation of contradictory results, stating:

This may be because of the fact that the respiratory chambers used in oxygen consumption work are of such a size as to produce a crowding effect upon the contained fishes, causing movements that would not otherwise occur. In such experiments the fishes were seldom quiet in the respiratory chambers, usually moving in vertical paths from bottom to top in the chambers. This was true both with isolated fishes and with groups of four. Under these circumstances the effect of quieting, as usually noticed in the groups, was lost as the results here indicate.

Our results confirm those obtained by Schuett (1934) and by Escobar, Minahan, and Shaw (1936) on the locomotor activity of isolated goldfishes and goldfishes in groups of four; and, volume comparisons excepted, a correlation has been established between oxygen consumption and locomotor activity.

It is appropriate here to compare the relative sensitivities of the Winkler chemical analysis used in determining the oxygen consumption and the observational technique used in determining the locomotor activity. In favor of the chemical technique it may be said that it detects two cases, B and C in Table 1, in which the oxygen consumption of the fishes in the group of two is significantly higher than that of the fishes in the group of four though, in general, the oxygen consumption of the two groups tends to be the same. The locomotor activity technique detects no difference in a similar comparison. Table 4 shows a significant increase in oxygen consumption of goldfishes placed in larger volumes, while Table 6 shows no corresponding effect on locomotor activity. Table 8 indicates a decreased oxygen consumption of fishes in "conditioned" water, while Table 9 shows no corresponding effect on the locomotor activity. In favor of the observational technique it may be said that it indicates more effectively in Table 7, comparing the isolated fish in 6 liters with the fishes in the group of two in 12 liters, that the isolated state may be sufficient to overcome the handicap of reduced total volume and to cause greater activity in the same volume of water per fish than does a similar comparison in Table 5 on oxygen consumption. Also, after Schuett (1934) had failed to obtain a "group effect" on oxygen consumption, using the modified chemical technique, he did get a "group effect" on locomotor activity, as did Escobar, Minahan, and Shaw (1936). However, the use by Schuett (1934) of Erlenmeyer flasks instead of aquariums for the tests on oxygen consumption must be borne in mind as a possible explanation. All things considered, it is the opinion of the writer that the chemical technique is the more sensitive one.

It is too early to attempt to discuss the broader implications of these and other studies on the mass physiology of the goldfish. In addition to the problems here at-

tacked, other workers in this laboratory have found (a) that when exposed to toxic amounts of colloidal silver, grouped goldfishes survive longer than those in isolation (Allee and Bowen, 1932); (b) that in an appropriate, simple setup, grouped goldfishes learn more rapidly than isolated animals (Welty, 1934); (c) that goldfishes consume more *Daphnia* per unit of time if grouped than if isolated (Welty, 1934); and (d) that under many experimental conditions small, isolated goldfishes grow more rapidly in water in which other goldfishes have lived than in uncontaminated water (Allee, Bowen, Welty, and Oesting, 1934; Allee, Oesting, and Hoskins, 1936; Livengood, 1937). In time, perhaps these and other studies may permit a critical examination of the general survival-value of numbers of goldfishes. For the present it is useless to do more than to recognize that such general problems exist and that they are not dissociated from the present studies.

SUMMARY

1. In a given volume of water an isolated goldfish consumes more oxygen and has a higher rate of locomotor activity than does each fish in a group of two.
2. In a given volume of water an isolated goldfish consumes more oxygen and has a higher rate of locomotor activity than does each fish in a group of four.
3. In a given volume of water each goldfish in a group of two tends to consume the same amount of oxygen and to have the same rate of locomotor activity as does each fish in a group of four.
4. Fishes in "conditioned" water exhibit the same type of "group effect" as do those in unconditioned water.
5. Fishes in "conditioned" water consume less oxygen than do those in unconditioned water.
6. Volume relationships are complex and require further analysis.
7. The modified Winkler technique appears to be more sensitive in determining oxygen consumption than the observational technique in determining locomotor activity.
8. A partial recheck of oxygen-consumption experiments, using manometric methods, confirms the relationships summarized in conclusion No. 2.

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AN ANALYSIS OF THE EFFECT OF NUMBERS UPON THE OXYGEN CONSUMPTION OF *CARASSIUS AURATUS*¹

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THE investigations reported here represent a continuation of research on an aspect of the mass physiology of animals—the effect of grouping on the activity of fishes (Shlaifer, 1938). Schuett (1934) found that an isolated goldfish, *Carassius auratus* Linné, was more active in an aquarium than were fishes in a group of four in the same volume of water. Escobar, Minahan, and Shaw (1936) confirmed these findings. Breder and Nigrelli (1938) also demonstrated the increased locomotor activity of *C. auratus* when isolated than when in a group of four. There remained, however, the conflicting reports of Schuett (1933) and Bowen (1932) as contrasted with the later work of Schuett (1934) concerning the relation between isolation and oxygen consumption in the goldfish. Schuett's later and more careful work indicated that there was no difference in the amount of oxygen used by a goldfish whether it was isolated or a member of a small group. Shlaifer (1938), measuring the activity of goldfishes indirectly through oxygen consumption and directly through observation, was able to resolve the conflict between studies on locomotor activity and studies on the use of oxygen. He found, among other things, that an isolated animal consumed more oxygen and had a higher rate of locomotor activity than did each fish in a group of two or of four.

The data in the present paper were collected in an attempt to analyze the factor or factors involved in the now well-established "group effect" in *C. auratus* and perhaps thereby to facilitate the analysis of the integrating mechanisms and behavior of fish aggregations in general.

MATERIALS AND METHODS

An extensive description of the general methods used may be found in a previous paper (Shlaifer, 1938). All experiments were performed in a room in Whitman Laboratory which has north windows only; the experiments were conducted between the hours of 9:00 A.M. and 12 noon. At the conclusion of a day's experiments, the fishes were placed in tap water, filtered free of chlorine and sediment, for a 21-hour period of acclimatization. The water was not changed until the conclusion of the experiments on the following day; this eliminated any disturbing effect of handling. Air, bubbled through the aquariums during acclimatization, maintained a normal oxygen tension. With one exception all the animals used were specimens of the common goldfish 5 cm. in length. The animals used for the experiments summarized in A of Table 1 were larger, measuring 8–10 cm. in length; these experiments were performed six months before those summarized in B of Table 1. The technique used throughout was the Rideal-Stewart modification of the Winkler method. The amount of oxygen used is a less direct indicator of activity than is the observation of locomotion. However, its measurement was deemed preferable for several reasons. In the first place, the observational method is not well adapted for sev-

¹ The writer is deeply grateful to Dr. W. C. Allee for his inspiring advice and helpful criticism.

eral of the types of experiments performed—e.g., darkness tests. In the second place, there is good evidence (Shlaifer, 1938) that the measurement of oxygen consumption is free from many of the sources of error that are inherent in the observational technique and is, in general, more sensitive. Possibly it reflects in time such subtle reactions as the movement of the fins or changes in the rate of heart beat caused by stimuli received from other members of the group. Since the activity of individual fishes may vary to some extent and since the activity of any one animal may also vary relatively from day to day, it was necessary to perform a fairly large number of experiments with a large number of animals in order to minimize this source of variation. This was especially true for the oxygen-consumption tests which preclude the measurement of the activity of any single fish in a group; it is also true for experiments utilizing the observational method, since this is less sensitive. In the past investigators along these lines have sometimes made generalizations hardly warranted by the data, considering the known sources of variation. When the oxygen consumption of isolated animals was compared with that of animals in a group of four, equal sampling was effected by performing four times as many experiments on the isolated fishes. These same isolated animals were later used in the groups of four.

EFFECT OF DARKNESS

Among the vertebrates, vision is a particularly important factor in the integration of aggregations, schools, and flocks of fishes and birds. Welty (1934) found that goldfishes become conditioned to run a simple maze more rapidly when grouped than when isolated. The group seemed to stimulate learning behavior through various ways which were all visually integrated. The importance of vision in the schooling of fishes has been shown by Parr (1927) for *Pneumatophorus grex* (Mitchill), by Breder (1929) for *Jenkinsia stolifera* (Jordan and Gilbert), by Spooner (1931) for *Morone labrax* Linnaeus, by Bowen (1931) who found that the closely schooling catfish, *Ameiurus melas* (Rafinesque), failed to aggregate in the dark, and by Breder and Nigrelli (1935) for *Lepomis auritus* Linnaeus.

If vision is a dominant factor in the effect of grouping on the respiratory activity of goldfishes, this effect should disappear in darkness. The experiments described below explore this possibility.

Methods.—Control tests were performed with fishes placed in isolation and in groups of four in ordinary daylight, free from sunlight. At the conclusion of a day's experiments the water was changed, and the aquariums were placed in a dark box which was known to be light tight. The oxygen consumption of the fishes in this experimental condition was measured in a three-hour test period after 21 hours of acclimatization. The same animals were acclimatized and tested for the same period of time in daylight as in darkness. Those tested in isolation were subsequently placed in groups of four, while those in groups of four were later isolated. In the experiments otherwise performed in darkness, the aquariums were removed from the dark box just prior to the test period in order to pour on the seal of mineral oil and to obtain several samples of water for oxygen analysis. This sudden change in illumination may have been a source of artificial stimulation, but was, of course, the same for isolated and grouped animals. An attempt was made to minimize this condition by drawing the shades of all windows and placing a dark cloth over each aquarium until the samples were obtained. Animals that were not in use were kept in a large aquarium in ordinary daylight or under general illumination from ceiling lights when these were on at night.

Results.—Reference to Table 1 shows that in both sets of experiments, A and B, in daylight, the oxygen consumption of the isolated animals is significantly higher than that of fishes in a group of four. This amply confirms previous experiments covering this point (Shlaifer, 1938). In total darkness, on the other hand, there is no significant difference. Thus, it is seen that the "group effect" disappears in the absence of light. Examining the data further, we find that in both A and B the oxygen consumption of fishes in a group of four is not significantly higher in daylight than in darkness. Isolated animals, however, have a distinctly higher rate of oxygen consumption in the light. Thus, the

TABLE 1
EFFECT OF DARKNESS ON THE AMOUNT OF OXYGEN CONSUMED
PER FISH BY GROUPED AND ISOLATED GOLDFISHES

EXPERIMENTAL CONDITION	TEST PERIOD (HOURS)	I. ONE FISH			IV. FOUR FISHES		
		No. of Cases	Mean Cc. O ₂ Con- sumed per Fish		No. of Cases	Mean Cc. O ₂ Con- sumed per Fish	
			Daylight	Darkness		Daylight	Darkness
A*, §, †.....	3	32×1	7.44	6.10	8×4	6.01	5.70
B§, †, ‡.....	3	32×1	3.82	2.90	8×4	2.72	2.60

* = 8-10-cm. fish.

† = In 7.5-liter aquarium.

‡ = In 6 liters of water.

§ = 5-cm. fish.

TABLE 1a
STATISTICAL ANALYSIS OF TABLE 1 SHOWN IN *P*-VALUES*

Experimental Condition	I vs. IV—Light	I vs. IV—Dark	I—Light vs. I—Dark	IV—Light vs. IV—Dark
A.....	0.026	0.590	0.0002	0.380
B.....	0.0048	0.406	0.0000	0.264

* Upper limit of statistical significance is set at 0.05. This is three times the probable error. *P* = 0.01 indicates good significance, while a value of 0.100 or more indicates little significance ("Student," 1925).

group effect appears to be eliminated not through increased use of oxygen by grouped fishes in the dark but by the decreased consumption of the isolated animals in that state.

These data indicate the importance of vision as a factor in the lessened activity of grouped goldfishes.

EFFECT OF BLINDNESS

If the group effect is lost in total darkness, indicating a role of vision, it follows that the same results should obtain with blinded fishes. Parr (1927) found that blinded individuals did not mill or exhibit schooling reactions. Bowen (1931) found that blinded catfishes did not aggregate.

Methods.—A group of individuals from set B of the darkness tests were blinded by completely removing both eyes. Though more drastic, this method seemed to be prefer-

able to covering the eyes with opaque materials which might wear off. Several animals did not survive the operation, and only those were used which, except for their blindness, exhibited normal behavior, at least as judged by the observer. The fishes that died did so in a few days; the survivors were observed for several weeks before they were used. These animals lived for months after the experiments were completed, indicating excellent recovery from the operation. The blinded fishes were placed alternately in isolation and in groups of four in daylight, and their oxygen consumption was measured in a test period each day. Removal of their eyes does not permit the use of the experimental animals as control animals every other day as was possible in the tests in darkness. However, it should be borne in mind that before being blinded, these animals had been used in the preceding series of experiments and, when in ordinary daylight, showed a definite group effect.

Results.—The data in Table 2 indicate that when fishes are blinded, there is no significant difference in the oxygen consumption of grouped and isolated animals. When com-

TABLE 2
EFFECT OF BLINDNESS ON THE AMOUNT OF OXYGEN CONSUMED
BY GROUPED AND ISOLATED GOLDFISHES*

ISOLATED		GROUP OF FOUR		MEAN DIFFERENCE	P
No. of Cases	Mean Cc. of O ₂ Consumed per Fish	No. of Cases	Mean Cc. of O ₂ Consumed per Fish		
28×1	2.20	7×4	2.07	0.13	0.7186

* Test period, 3 hours; volume, 6 liters of water in a 7.5-liter aquarium.

pared with the fishes placed in darkness in B of Table 1, the blinded animals, themselves selected from set B, are seen to have a lower rate of oxygen consumption in both the isolated and the grouped states. This is partially a result of the fact that the surviving blinded animals were somewhat smaller than those in the original group. Thus, the elimination of the action of vision through blinding also eliminates the group effect.

VISUAL CONTACT

Though the darkness and blindness experiments show that the sense of vision is important, they do not rule out the possibility of other sense organs coming into play in the group behavior of the goldfish in an important though perhaps subtle manner. It is possible that vibrations of tails of fishes set up pressure disturbances, detected perhaps by the lateral-line sense organs, which influence the activity of grouped animals. Thus, in the strongly thigmotactic *A. melas* Bowen (1931) demonstrated such responses and touch as well in the aggregating behavior of this species. Even with these bullheads vision was found to be essential for aggregations since blinded fishes and normal ones kept in the dark did not aggregate.

The experiments reported below were designed to eliminate all factors but vision. If isolated goldfishes manifest a group effect when in contact through vision alone with

other goldfishes, it may be concluded that vision is a very important factor in their normal group behavior.

Methods.—A 3.5-liter aquarium with dimensions of 17.5 cm. by 13 cm. and 20 cm. deep was placed in a 15-liter aquarium whose dimensions were 30 cm. by 27 cm. and 24 cm. deep. The walls of both aquariums were of transparent glass. The smaller aquarium was filled with 2.5 liters of water which gave a depth of 13 cm. at the mark. The larger aquarium was filled with enough water, about 6 liters, so that, when the smaller one was placed in it, the two water levels coincided. On each of the four vertical sides there were 5 cm. of space between the inner surface of the large aquarium and the outer surface of the small aquarium. One goldfish was placed in the smaller aquarium. In the control tests there were no animals in the outer aquarium, while in the experimental condition there were three goldfishes present. The animals used in all experiments were rather small—5 cm. in length; this permitted sufficient free movement in either aquarium. The usual oxygen seal of mineral oil was placed on the water surface of

TABLE 3
EFFECT OF VISUAL CONTACT WITH OTHERS OF THE SAME SPECIES ON THE
AMOUNT OF OXYGEN CONSUMED BY ISOLATED GOLDFISHES*

CONTROL†		EXPERIMENTAL‡		MEAN DIFFERENCE	P
No. of Cases	Mean Cc. of O ₂ Consumed	No. of Cases	Mean Cc. of O ₂ Consumed		
16	1.99	16	1.32	0.67	0.0002

* Test period, 3 hours; volume, 2.5 liters of water in a 3.5-liter aquarium.

† Isolated fish in no visual contact with other fishes.

‡ Isolated fish in contact through vision alone with three other fishes.

the small aquarium only. The oxygen consumption of the isolated animal in the small aquarium was measured each day. The isolated fish was alternated daily between complete isolation and contact through vision alone with three other animals of the same species. Several fishes were tested in the course of the experiments. One might be inclined to doubt whether the optical powers of the goldfish are sufficiently acute to enable the isolated animal in the foregoing setup to respond to its fellows in the outer aquarium. However, Welty (1934) in the course of his experiments on learning in the goldfish showed that these animals learn to swim a simple maze more rapidly after having seen, through transparent glass, other fishes perform in it.

Results.—Table 3 shows a significant decrease in the oxygen consumption of the isolated fish in the inner aquarium when in visual contact with three others of its kind.

Since all other stimuli but those due to vision have been eliminated, this experiment indicates that visual response to other fishes is a dominant factor responsible for the group effect in goldfishes which is indicated by the decreased rate of activity and oxygen consumption in a group of four as contrasted with similar data for isolated animals.

EFFECT OF MIRROR IMAGES

This experiment was designed to investigate further the role of visual stimuli in aggregations of *C. auratus*. Also, it furnishes an interesting sidelight on general fish be-

havior. Spooner (1931) found that the bass—*Morone labrax*—a vigorous fish with a rather strong tendency to school, gave definite reactions to a mirror. An isolated fish in contact with a mirror would often lie motionless against it for extended periods of time. When Spooner (1931) used mirror surfaces that were broken up by running elastic bands across the surface of the mirror at definite intervals, the fishes reacted distinctly to these mirrors if the bands were three-quarters of an inch apart; at smaller intervals there were either weak reactions or none at all.

Methods.—7.5-liter aquariums containing 6 liters of water were used. The aquariums used for the control tests had four transparent vertical glass sides; those used for the experimental condition were mirrored on two contiguous vertical sides which formed a right angle. The remaining two vertical sides of the mirrored aquariums, also forming a right angle, were constructed of transparent glass. Isolated animals were alternated each day between the plain and mirrored aquariums, and their oxygen consumption was measured in a 3-hour test period.

TABLE 4
EFFECT OF MIRROR IMAGES ON THE AMOUNT OF OXYGEN CONSUMED
BY ISOLATED GOLDFISHES*

CONTROL†		EXPERIMENTAL‡		MEAN DIFFERENCE	P
No. of Cases	Mean Cc. of O ₂ Consumed	No. of Cases	Mean Cc. of O ₂ Consumed		
22	2.92	22	2.09	0.83	0.0000

* Test period, 3 hours; volume, 6 liters of water in a 7.5-liter aquarium.

† Isolated fish in a clear-sided aquarium.

‡ Isolated fish in an aquarium mirrored on two adjacent sides.

Results.—The data in Table 4 show a significant fall in the oxygen consumption of the isolated fish when in the mirrored aquarium. All factors but vision have again been eliminated. The animal in the mirrored aquarium tended to remain near and parallel to the mirrored surfaces, apparently reacting as though it was lining up with another fish in a group of two.

The experiments described above leave little doubt as to the important role played by sight in these group reactions and make unnecessary the analysis of other factors at this time. Granted that this sense is so important in the responses tested, how does it act? The fishes may be reacting to (a) color, (b) form, (c) type of movement, (d) amount of movement, or (e) some combination of these factors.

REACTION TO COLOR

The response of fishes to colors is a field which still requires investigation. In a comprehensive review Warner (1931) criticized the lack of control of the intensity factor and was of the opinion that most of the experimental work would bear repeating. White (1919, 1927) demonstrated that mud minnows and sticklebacks can discriminate wavelengths and not merely intensities of light. Noble and Curtis (1935) found that when the female cichlid, *Hemichromis bimaculatus*, was given a choice between a male with cry-

throphores expanded by yohambine and a paler control, she usually selected the former. Brown (1937) demonstrated the reaction of the largemouthed black bass, *Aplites salmoides*, to colors.

Methods.—The aquarium setup was the same as that described for the visual-contact experiments. In the control setup three normal orange-colored goldfishes were placed in the large aquarium. In the experimental condition, these animals were replaced by three goldfishes of the same size and shape and of a dark gray or black color, mixed with a slight trace of orange. In general, at least to the human eye, the experimental animals formed a distinct contrast to those used in the control tests. Isolated normal orange-colored goldfishes in the smaller aquariums were alternately placed in visual contact with the orange and with the dark animals, while their oxygen consumption was measured as usual.

Obviously, these experiments were not designed to test the reactions of *Carassius* to color as such. Rather, they are naturalistic in nature and do explore the possibility that orange goldfishes respond differentially to differently colored members of their species.

TABLE 5
EFFECT OF VISUAL CONTACT WITH ORANGE OR WITH DARK INDIVIDUALS
ON THE OXYGEN CONSUMPTION OF ISOLATED GOLDFISHES*

CONTROL†		EXPERIMENTAL‡		MEAN DIFFERENCE	P
No. of Cases	Mean Cc. of O ₂ Consumed	No. of Cases	Mean Cc. of O ₂ Consumed		
24	1.71	24	1.61	0.10	0.363

* Test period, 3 hours; volume, 2.5 liters of water in a 3.5-liter aquarium.

† Isolated fish in visual contact with three normal orange goldfishes.

‡ Isolated fish in visual contact with three darkly colored goldfishes.

Results.—Reference to Table 5 shows that there is no significant difference in the rate of oxygen consumption of an isolated fish when it is in visual contact with a goldfish of a distinctly darker color than the normal orange type. Thus, the major visual response of fish to fish is apparently not in terms of color. It may be that the technique and experimental setup were not sensitive enough, but the general indications are that other factors are involved.

REACTION TO BODY FORM AND MOVEMENT

The factors that remain to be analyzed are the reactions to body form and to movement. Spooner (1931) found that a fish will be attracted to and will lie alongside a dead fish supported by glass in the position of a resting live one. Thus it is seen that a fish may be attracted by the form of another one that is completely devoid of movement. Apparently, the form in that case had to be a good replica of the living animal, for, when rough models of fishes were tried, no attraction was obtained.

The following experiment was designed to test the factors of form and movement simultaneously.

Methods.—The aquarium setup was again the same as that described for the visual-contact tests. Normal orange goldfishes were killed and injected with formaldehyde and

were mounted on sharply pointed glass rods attached at the base to hidden wire loops for support. These mounted animals were placed in the outer aquarium in the normal swimming position close to and parallel to the sides of the smaller aquarium. The fishes in both aquariums in that type of setup usually swim in a horizontal plane about 4 cm. above the bottom. Accordingly, the mounted animals were also placed at that level. No odor of formaldehyde, which had been injected into the dead animals, could be detected when they were in position in the aquarium. Three mounted fishes were placed in the larger aquarium in the experimental setup, two of them, nose to tail, next to one long side of the small aquarium; the third was placed in the opposite passage way and pointed in the same direction as the other two. When it was thought that the color of the mounted specimens was not quite normal, they were replaced by newly killed and mounted animals. The hollow transparent glass rods on which the fishes were mounted probably eliminated any possibility that the animal in the inner aquarium would respond to the mounting material as well as to the mounted fish. The isolated test animals in the inner

TABLE 6
EFFECT OF VISUAL CONTACT WITH NORMAL OR WITH MOUNTED INDIVIDUALS ON THE OXYGEN CONSUMPTION OF ISOLATED GOLDFISHES*

CONTROL†		EXPERIMENTAL‡		MEAN DIFFERENCE	P
No. of Cases	Mean Cc. of O ₂ Consumed	No. of Cases	Mean Cc. of O ₂ Consumed		
20	1.95	20	1.46	0.49	0.0001

* Test period, 3 hours; volume, 2.5 liters of water in a 3.5-liter aquarium.

† Isolated fish in visual contact with three normal goldfishes.

‡ Isolated fish in visual contact with three dead and mounted goldfishes.

aquariums were alternately placed in visual contact with the control setup of three normal orange goldfishes and with the experimental condition of the three killed and mounted specimens; their oxygen consumption was measured as usual in a 3-hour test period.

Results.—The data in Table 6 show that the oxygen consumption of the isolated fish is significantly lower when that animal is in visual contact with immobilized fishes. The interesting implications of this experiment are too lengthy to be treated here and are reserved for the following section.

DISCUSSION

The effect of grouping on the oxygen consumption and locomotor activity of fishes is an aspect of animal aggregations. Accordingly, as a somewhat minor result of these experiments, it is possible to suggest the place occupied by *C. auratus* in a system of classification of such aggregations. This becomes more interesting in connection with the fact that "... no hard and fast line can be drawn between well-integrated social organizations and loosely integrated aggregations which are usually regarded as being definitely non-social" (Allee, 1931, p. 14), since the goldfish is not a closely schooling species. Presumably, therefore, it occupies a low position even in the more inclusive estimates of the extent of sociality such as those given by Wheeler (1928) and Allee (1938).

In the system of classification of animal aggregations given by Allee (1931, p. 35, 36), the aggregating behavior of the goldfish might conceivably fall into the two major divisions given for individuals which are not organically connected: (1) aggregations primarily due to reactions to the environment and (2) aggregations primarily due to reactions to other organisms.

Theoretically, goldfish aggregations that fall into the first category may occur in nature. Thus, the animals may be attracted tropistically to some particular region of a pond that at a particular time presented optimal chemical or physical conditions. This type of aggregation is much less likely even in large aquariums or tanks in the laboratory; in smaller aquariums under controlled experimental conditions, it may practically be eliminated from consideration. For example, it is observed that goldfishes in a group of four may tend to occupy, more or less, a certain region in an aquarium, the region occupied being changed from time to time. When the animals are blinded, they are distributed at random indicating that the reaction was a result of integration with other individuals rather than caused by some differential factor in the environment.

Aggregations of goldfishes in nature probably fall much of the time in class 2; in laboratory aquariums, properly protected from any changes in the uniformity of the environment such as sunlight, proximity to heat, etc., category 2 would be the sole possibility.

In addition to the foregoing subdivisions of aggregations based on the method of integration, Allee (1931, p. 36) lists the following subdivisions based on the degree of integration:

1. Relatively slightly integrated groups in which the primary (individual) reactions predominate and whose survival value is apparent only after experimentation.
2. Moderately well-integrated groups in which the secondary (group) reactions predominate, although primary reactions are still strongly in evidence, and whose survival value is more obvious.
3. Highly integrated groups in which the primary reactions are decidedly in the minority and the social value is strongly in evidence.

Aggregations of *C. auratus* fall nearer to class 1 than to class 2. The closely schooling mackerel would fall definitely into class 2.

Shlaifer (1938) found that a goldfish has a higher rate of oxygen consumption and locomotor activity when isolated than when it is a member of a freely moving group. The experimental data presented here confirm these earlier results and extend them to demonstrate the importance of visual stimuli in decreasing the activity of grouped goldfishes. Visual contact of an isolated animal with freely swimming goldfishes or even with a mirror self-image retards activity. This is found to be independent of the color. The data listed in Table 6 also show that the activity of the isolated animal is greater if the fishes in the visual field are moving than if they are immobile. Therefore, the isolated goldfish in visual contact with the group of immobilized animals is affected in its behavior even more than if the fishes in its visual field are active. Under these conditions the isolated animal can be reacting to form and to color only. Since color, within the limits tested, is not needed to produce the usual group effect, it appears that, under the conditions tested, the isolated fish is reacting to form alone. Apparently, it is enough that a goldfish see its own mirror image or an immobilized goldfish in order to undergo the quieting effect of the group. The extent to which this form can be altered from that of goldfishes and still evoke a similar effect remains to be discovered by direct experimentation.

It was shown in the darkness experiments that the group effect is eliminated not

through an increase in oxygen consumption by the grouped fishes in the dark but by decreased consumption by isolated animals in that state. If we assume that darkness tends to act as a quieting factor for goldfishes, an explanation may be offered for the observed results as follows: The isolated animal, subjected to no stimuli from other fishes, is affected only by the change in illumination, and its activity is diminished; in the grouped animals, the quieting effect of darkness is equalized by the loss of visual inhibition of activity and, perhaps, by random contacts.

The dominance of vision as the factor that inhibits activity in groups of goldfishes can be further confirmed by comparing certain data in Tables 1 and 3. These experiments were selected for comparison because they are the only ones whose general conditions are similar enough for that purpose. Even these experiments were performed under setups that were sufficiently different to make impossible a perfect comparison. Thus, the oxygen consumption of the fishes in Table 3 is lower, regardless of the condition of grouping, than is that of the animals in B of Table 1; this is probably due to the distinctly lower volume of water available to the animals in the smaller aquarium used for the experiments summarized in Table 3. (A of Table 1 is not used in the comparison because the individuals in those experiments were considerably larger than those used later.) It is seen in B of Table 1 that the oxygen consumption of an isolated fish in daylight is decreased by 29 per cent when it is subsequently used as a member of a group of four. Reference to Table 3 shows that, when an isolated fish is made a member of a group of four through visual contact alone, its oxygen consumption is reduced by a similar amount—33 per cent. Thus, visual response to other fishes, taken alone, may produce a group effect of the same order of magnitude as does that shown when an individual is an actual member of a group of four.

Of the four factors involved in visual response—namely, color, form, type of movement, and amount of movement—only form has been found to act as an inhibitor of activity. The data in Table 6 indicate that inhibition of activity of an isolated goldfish is greater when it responds through sight to form *minus* movement than it is when the animal responds to form *plus* movement. This may mean that movement as such is a stimulator of activity. The role of the type of movement in aggregations of fishes is a much more subtle affair and may be investigated through the use of heterotypic groups.

Experiments on fishes in laboratory aquariums suffer from the criticism of being divorced from conditions as they occur in nature. However, the obvious necessity for the careful control of variables greatly mitigates this criticism. Apropos of this consideration, the experiments described in this paper may be listed in the following increasing degree of artificiality: (a) experiments with normal fishes in darkness, (b) experiments with normal fishes in daylight, (c) experiments with blinded fishes, (d) effect of mirror images, and (e) contact through vision alone with (1) normal fishes and (2) mounted fishes.

Many reactions of animals tested experimentally may never occur, as far as we know, in a natural environment. Nevertheless, it is important to know not only the normal behavior that usually is manifest but also the behavior potentialities of an organism. Very often it is the analysis of behavior under unnatural conditions which are carefully controlled that facilitates the explanation of normal behavior. Also, many factors in the behavior complex of an organism are so subtle as to necessitate artificial methods of analysis if they are to be discovered at all.

As discussed above, it is apparently the visual perception of body form that inhibits

the activity of grouped goldfishes. Granted that this is so, it is difficult to offer an explanation for this pattern of behavior. Breder and Nigrelli (1935) state that a schooling fish primarily holds a position of maximum "comfort" by fixing its vision on some object, either a stationary one or a moving one; this difference determines whether the fish maintains a stationary position or swims forward. If this also holds true for the goldfish, which is not a strongly schooling animal, it may be that the increased activity of the isolated animal, which cannot find an object on which to fix its vision, is a result of a state of "psychic unrest," a state which may well defy direct experimental exploration. Thus, as in most aspects of animal behavior, the ultimate analysis of the *modus operandi* remains as elusive as it is interesting.

One may speculate as to whether the reaction of a goldfish to the body form of others in a group is a response to the particular type of body form characteristic of the species or to body form of fishes in general. Escobar, Minahan, and Shaw (1936) worked with a heterotypic group of four fishes; the species used were *C. auratus*, *Gambusia affinis*, *Oryzias latipes*, and *Macropodus verdi-auratus*. The group effect found here for the goldfish was the opposite of that found for it in homotypic groups of four. The activity of the goldfish in the heterotypic group exceeded even that in an isolated condition. More extensive work with heterotypic groups in observing locomotor activity and measuring oxygen consumption and utilizing, perhaps, some of the experimental setups described above may yield fruitful and interesting results which would have significance for the general problem of the integration of animal aggregations. It is quite probable that any group effect that might be found in such heterotypic groups would be produced by a combination of factors distinctly more complex than is to be found in the homotypic groups used so far. Thus, the color, form, type of movement, and amount of movement might vary considerably when several species are used. The activity of a goldfish in heterotypic groups of three species might in some cases be found to be a resultant of two opposing tendencies caused by the other two species in the group. Perception of body form might stimulate activity in heterotypic groups, or its inhibitory action might be masked by some more dominant responses to type and amount of movement. It would be necessary, of course, to employ suitable techniques to isolate, as far as possible, the variables in this complex. Any conclusions drawn from the behavior of fishes in heterotypic groups would have to be based on a considerably larger group of data than is necessary for homotypic groups because of the variables involved. However, the implications, especially at the relatively low state of integration that is involved in such aggregations, appear to justify the work that would be necessary. Such an analysis is the more important because heterotypic aggregations have ecological implications of far-reaching significance in the study of animal communities in nature. Experiments along these lines are now under way in our laboratory.

Parr (1937) makes the interesting point that a fish with a rather rigid body—the mackerel, for instance—displays definite schooling behavior visually integrated, though it is incapable of self-perception. Therefore, Parr (1937) states: "... the reaction of the mackerel to the perception of others of its kind cannot be in terms of a response to individuals recognized to be similar to itself, but must be based upon an automatic association mechanism of a different order in which the perception of another fish similar to itself fits as a key fits into a lock." If this is true for the mackerel, it also holds for *C. auratus*. From this point of view, the proposed analysis of heterotypic aggregations of fishes presents new and interesting problems.

SUMMARY

1. The effect of numbers upon the oxygen consumption of grouped and isolated goldfishes, manifested by a decreased rate of oxygen use in the group, is lost when the animals are placed in the dark or are blinded.
2. The oxygen consumption of isolated goldfishes is decreased when they are placed in contact through vision alone with others of the same species, with their own mirror images, or with recently killed and mounted forms.
3. It is evident that vision is the major sense involved in this aspect of mass physiology.
4. Inhibition of activity in grouped animals is probably due to the perception of the form rather than the color or movement of the fishes comprising the group.

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